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Telomere Length in Myelodysplastic Syndromes

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We have studied telomere length in the bone marrow cells or the granulocyte and lymphocyte cell fractions of 54 patients with myelodysplastic syndromes (MDS) by Southern blot hybridization using the $(TTAGGG)_4$ probe. The average telomere length expressed as the peak telomere repeat array (TRA) in the peripheral blood, or bone marrow samples obtained from a group of 21 healthy age-matched controls (26–89 years old, mean age 55), ranged between 7.5 and 9.5 kb (mean peak TRA 8.6 kb). Twenty-four patients with refractory anemia (RA) were studied; 10/24 (42%) had telomere reduction (<7.5 kb) relative to age-matched controls and the mean peak TRA was 7.5 kb (range 4.0–9.0 kb). Eleven patients with RA with excess blasts (RAEB) were studied; 5/11 (45%) had reduced telomeres relative to age-matched controls and the mean peak TRA was 7.1 kb (range 5.0–9.0 kb). Eighteen patients with MDS in transformation to AML, comprising 15 with RAEB in transformation (RAEBt) and 3 with CMML in transformation (CMMLt), were also studied. Thirteen of eighteen patients (72%) had telomere reduction relative to age-matched controls and the mean peak TRA was 6.1 kb (range 3.5–9.0 kb). Thirty-six patients included in the study had either a normal karyotype or a simple karyotype (1 karyotypic change) and 20/36 (55%) of these had telomere reduction and the mean peak TRA was 7.1 kb (range 4.3–9.0 kb); 8 patients had a complex karyotype (3 or more karyotypic changes) and 5/8 (62%) of these had telomere reduction and the mean peak TRA was 6.1 kb (range 3.5–9.0 kb). We conclude, firstly that there is heterogeneity of telomere length in MDS and that this is observed throughout the spectrum of FAB-subtypes. Secondly, these data show that a marked reduction in telomere length in MDS if often associated with leukemic transformation and with the presence of complex karyotypic abnormalities. Am. J. Hematol. 56:266–271, 1997.

Key words: leukemia; MDS; telomere

INTRODUCTION

Telomeres are the termini of eukaryotic chromosomes and are composed of a series of simple tandem repeat sequences [1]. The repeated sequences (TTAGGG)n have been highly conserved throughout evolution and in humans 10–15 kb of TTAGGG repeats cap the chromosome ends [2]. Telomeres have a number of important functions [3]. There is evidence to suggest, for example, that telomeres stabilize the chromosome ends and protect them from degradation, and recombination [1,3]. It is also probable that telomere proteins function in the positioning of telomeres and of chromosomes in the nucleus [4]. Much interest has centered recently on studies that have suggested that telomeres and the enzyme telomerase [5–7] may have a key role in the regulation of cell life span [8,9]. A number of studies have shown that telomeric DNA is lost, both in vitro and in vivo, each time human cells divide [10–12]. Indeed, human telomeres progressively shorten with age and it has been suggested that the reduction in telomeric length may function as a mitotic clock controlling the number of times a cell can divide [8,13,14].

Reduction in telomere repeat length has been observed in a number of human malignancies [15,16] including

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both solid tumors [9,17–19] and leukemias [20–22]. Whilst a reduction in telomere length is a feature of many human malignancies, it is interesting to note that the majority of late stage human tumors express telomerase [23,24]. It is now thought that this apparent paradox may be explained by such tumors having multiplied to such an extent that telomerase is essential to survival, i.e., it is required to stabilize telomere length and to halt the loss of further telomere DNA [23,25].

There is much interest in telomere length in hemopoietic malignancy [20–22], particularly with regards to disease progression [25,26]. The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal myeloid disorders [27,28] and comprise refractory anemia (RA), RA with excess blasts (RAEB), RAEB in transformation (RAEBt), RA with ringed sideroblasts (RAS), chronic myelomonocytic leukemia (CMML), and CMML in transformation (CMMLt) [29]. MDS is characterized by dyshematopoiesis and/or impaired maturation of hemopoietic cells [27,28]. Although the bone marrow is typically hypercellular, the peripheral blood is often characterized by cytopenia [27,28]. Approximately 50% of MDS patients have karyotypic abnormalities [30,31]. MDS patients have frequent evolution (20–40%) to acute myeloid leukemia (AML) and there is interest in the determination of those factors that may lead to leukemic transformation [32–35]. Ohyashiki et al. have carried out an investigation into telomere length in MDS and have reported some correlation with telomere reduction and poor prognosis [26]. We have investigated telomere length in a large group of patients with MDS and have found, firstly, that there is marked heterogeneity of telomere length in MDS and, secondly, that telomere shortening is often associated with transformation to AML and with the presence of complex karyotypic abnormalities.

MATERIALS AND METHODS

Patients

Fifty-four patients with MDS were included in the study. Classification was according to the French-American-British (FAB) criteria [29] and the patients were selected solely on the basis of a diagnosis of MDS. At the time of investigation 22 patients had RA, 2 RAS, 1 CMML, 11 RAEB, 15 RAEBt, and 3 had CMMLt. The age range of the 54 patients at diagnosis was 26–88 years and the mean age was 66 years. Chromosome preparations were obtained from bone marrow samples using standard techniques [36]. Cultures were harvested after 24 h with a 1-h or overnight exposure to colcemid, and at 48 h after thymidine synchronization. All preparations were banded and the karyotype defined according to the International System for Human Cytogenetic Nomenclature, 1978. A minimum of 20 metaphases were karyotyped in each case.

Samples

Bone marrow samples were obtained from bone marrow aspirates, from all 54 of the MDS patients. In addition, fractionated peripheral blood leukocytes were obtained (mononuclear cells and granulocytes) from 16 of the patients. In these cases, mononuclear cells and granulocytes were separated from 40 ml of EDTA-treated patient peripheral blood by Ficoll gradient centrifugation [37]. The granulocyte fraction showed a high level of purity (>95%). T-lymphocyte populations (>90%) were isolated from the mononuclear fraction by erythrocyte rosetting [38]. The T-lymphocyte population is not part of the malignant clone in this group of MDS patients (data not shown) and, therefore, acts as an internal control. Peripheral blood samples, granulocyte cell fractions, or bone marrow samples from 21 normal healthy age-matched controls (age range 26–89 years, mean age 55 years) were also obtained.

Telomeric Repeat Analysis

High molecular weight DNA was obtained by phenol/chloroform extraction using standard methods [39] from bone marrow samples or fractionated peripheral blood leucocytes from the 54 patients with MDS and from the bone marrow samples (3), peripheral blood leucocytes (12), and fractionated peripheral blood leucocytes (6) from normal healthy individuals (age-matched controls). To ensure that there was no evidence for DNA degradation, the integrity of each of the undigested DNA samples was checked by electrophoresis through 1% agarose gels [39[. Ten micrograms of DNA digested with the restriction enzyme *Hinf*I was size-fractionated by electrophoresis through 0.8% agarose gels. The DNA was transferred to Hybond N (Amersham Int., Amersham, UK) according to standard procedures for Southern blotting [39]. The filters were prehybridized in 5 × SSC, 4 × Denhardts' solution 0.5% SDS and 100 μg/ml denatured salmon sperm DNA for 2–4 h at 65°C [20]. The filters were hybridized to a 3′-^{32}P labelled $(TTAGGG)_4$ telomeric probe in 5 × SSC for 16–24 h at 50°C [20]. The filters were washed in 4 × SSC for 30–60 min and autoradiographed between intensifying screens at −70°C for 2–7 days [20]. The telomere lengths were measured with an LKB Ultrascan XL densitometer (LKB, Bromma, Sweden), with the peak of telomere length in kilobases (peak telomere repeat array; peak TRA) taken as the average telomere length in each patient [26]. The telomeric repeat analysis was performed with each DNA sample on at least two separate occasions. After stripping, the same filters were rehybridized under standard conditions [39] with the probe BLUR-8 (Oncor Inc., Gaithersburg, MD), a human Alu I repeat sequence.

TABLE I. Peak TRA (kb) of Various Hematological Samples Obtained From a Group of 21 Age-Matched Healthy Controls*

Case no.	Age	Sample	Peak TRA (kb)
1	85	PB	8.0
2	81	PB	7.5
3	86	PB	8.5
4	85	PB	7.5
5	89	PB	8.0
6	60	PB	7.5
7	72	PB	9.0
8	72	PB	9.5
9	69	PB	9.5
10	69	PB	8.0
11	40	PB	8.0
12	29	PB	8.0
13	33	PB	9.5
14	40	BM	9.5
15	55	BM	9.3
16	55	BM	9.4
17	29	G	9.0
18	25	G	9.0
19	29	G	8.0
20	29	G	9.0
21	26	G	9.0

PB, DNA obtained from peripheral blood sample; BM, DNA obtained from bone marrow sample; G, DNA obtained from granulocyte cell fraction.

RESULTS

The peak TRA of the peripheral blood samples, granulocyte cell fractions, and bone marrow samples of a group of 21 healthy age-matched controls ranged between 7.5 and 9.5 kb and the mean peak TRA was 8.6 kb (Table I; see also Fig. 2). These control values are consistent with data from other groups concerning telomere length in human leukocytes within this age range (26–89 years) [9].

Fifty-four patients with MDS were included in the study. FAB sub-type, karyotype, samples analyzed, and the peak of telomere length in kilobases (kb) as the average telomere length are shown in Tables II and III (see also Figs. 1 and 2). The lowest TRA value observed in age-matched controls was 7.5 kb. A TRA of less than 7.5 kb was, therefore, regarded as a reduction in telomere length. Twenty-four patients with RA were studied (including 2 with RAS); 10/24 (42%) had telomere reduction (<7.5 kb) and the mean peak TRA was 7.5 kb (range 4.0–9.0 kb). Eleven patients with RAEB were studied; 5/11 (45%) had telomere reduction and the mean peak TRA was 7.2 kb (range 5.0–9.0 kb). One patient with CMML was studied and had a TRA of 7.0 kb. Fifteen patients with RAEBt were studied; 10/15 (66%) had telomere reduction and the mean peak TRA was 6.3 kb (range 3.5–9.0 kb). Three patients with CMMLt were studied; 3/3 (100%) had telomere reduction and the mean peak TRA was 4.9 kb (range 3.5–7.0 kb). Therefore, a combined total of 18 patients with MDS in transformation to AML were studied (15 RAEBt, plus 3 CMMLt); 13/18 (72%) had telomere reduction (<7.5 kb) and the mean peak TRA was 6.1 kb (range 3.5–9.0 kb). Thirty-six patients included in the study had either a normal karyotype or a simple karyotype (1 karyotypic change) and 20/36 (55%) of these had telomere reduction and the mean peak TRA was 7.1 kb (range 4.3–9.0 kb). Eight patients had a complex karyotype (3 or more karyotypic changes); 3 with RA, 3 with RAEB, and 2 with RAEBt. Five out of eight (62%) of these patients had telomere reduction and the mean peak TRA was 6.1 kb (range 3.5–9.0 kb). When the same filters were stripped and hybridized to the Alu I repeat probe no differences between the MDS samples and the control samples were observed (data not shown).

DISCUSSION

We have examined telomere length in a large group of patients with MDS. DNA obtained from the bone marrow samples or the fractionated peripheral blood samples of 54 patients with MDS was subjected to telomeric repeat analysis. Southern blot analysis using the $(TTAGGG)_4$ telomeric probe was performed. The results of this study show that there is marked heterogeneity of telomere length in MDS. Approximately 50% of patients with MDS showed a reduction in telomere length (<7.5 kb) relative to age-matched controls. This finding is consistent with some earlier observations made by Ohyashiki et al. in 1994 [26] and Counter et al. in 1995 [25]. Ohyashiki et al. studied telomere length in the bone marrow samples of 16 patients with MDS and concluded that telomere length appeared variable in MDS [26]. Furthermore, Counter et al. examined telomerase activity in 5 patients with MDS and suggested that telomerase is variably activated in MDS [25]. In contrast, it has been suggested that a reduction in telomere length always occurs in the blast cell population of patients with AML [20,25]. A study by Yamada et al., for example, demonstrated variable (2.7 to 6.4 kb) but consistent reduction in telomere length in all 7 cases of AML examined [20]. Similarly, Counter et al. demonstrated the presence of short telomeres (3.6–6.5 kb) in all 7 cases of AML investigated [25].

One of the aims of this present study was to determine whether the reduction in telomere length observed in some MDS patients correlated with any particular features of this myeloid disorder. Unfortunately, telomerase levels were not measured since this was a retrospective study of DNA samples. Ten of 24 (42%) patients with RA showed telomere reduction (<7.5 kb) and the mean peak TRA was 7.5 kb. From this study we are not able to comment whether telomere length in RA correlates to prognosis. Larger numbers of patients need to be studied prospectively to answer this important question. Thirteen

TABLE II. Peak TRA (kb) and Clinical and Cytogenetic Details of a Group of Patients With MDS*

Patient no.	FAB type	Karyotype	Sample	Peak TRA (kb)
1	RA	S	BM	6.8
2	RA	N	BM	5.3
3	RA	N	BM	8.0
4	RA	ND	BM	9.0
5	RA	S	BM	8.0
6	RA	2	BM	7.5
7	RA	N	BM	7.0
8	RA	C	BM	9.0
9	RA	N	BM	8.0
10	RA	ND	BM	7.5
11	RA	S	BM	9.0
12	RA	C	BM	4.0
13	RA	C	G	5.8
			L	9.0
14	RA	2	G	8.0
15	RA	N	G	8.0
			L	8.0
16	RA	N	G	7.0
17	RA	S	G	8.6
			L	8.6
18	RA	S	G	8.6
			L	9.0
19	RA	S	G	8.6
			L	9.0
20	RA	S	G	9.0
			L	9.0
21	RA	S	G	6.0
			L	8.0
22	RA	S	G	7.0
			L	9.0
23	RAS	S	G	7.0
24	RAS	N	G	7.0
25	RAEB	C	BM	9.0
26	RAEB	S	BM	7.0
27	RAEB	C	BM	8.0
28	RAEB	S	BM	8.0
29	RAEB	N	BM	7.5
30	RAEB	N	BM	6.4
31	RAEB	N	BM	7.5
32	RAEB	S	BM	7.5
33	RAEB	N	BM	5.8
34	RAEB	C	BM	5.0
35	RAEB	N	G	7.0
			L	8.0
36	RAEBt	N	BM	8.0
37	RAEBt	N	BM	5.0
38	RAEBt	2	BM	5.5
39	RAEBt	N	BM	6.5
40	RAEBt	N	BM	5.5
41	RAEBT	C	BM	5.0
42	RAEBt	ND	BM	4.0
43	RAEBt	ND	BM	8.0
44	RAEBt	N	BM	9.0
45	RAEBt	N	BM	5.0
46	RAEBt	ND	BM	9.0
47	RAEBt	N	BM	8.0
48	RAEBt	N	BM	5.5
49	RAEBt	C	G	3.5
			L	9.0
50	RAEBt	S	G	7.0
51	CMML	N	G	7.0
52	CMMLt	2	BM	7.0
53	CMMLt	ND	BM	3.5
54	CMMLt	N	BM	4.3

*PB, DNA obtained from peripheral blood sample; BM, DNA obtained from bone marrow sample; G, DNA obtained from granulocyte cell fraction; L, DNA obtained from T-lymphocyte cell fraction; N, normal karyotype; S, simple karyotype (one karyotypic abnormality); 2, 2 karyotypic abnormalities; C, complex karyotype (3 or more karyotypic abnormalities); ND, not done.

TABLE III. Average Peak TRA Values in Kilobases (kb) of a Group of Controls and Patients With MDS

Sample	Sample no.	Mean peak TRA (kb)	Median peak TRA (kb)	Peak TRA range (kb)	No. peak TRA <7.5 (kb)
Normal	21	8.6	9.0	7.5–9.5	0 (0%)
RA	24	7.5	7.7	4.0–9.0	10 (42%)
RAEB	11	7.2	7.5	5.0–9.0	5 (45%)
RAEBt	15	6.3	5.5	3.5–9.0	10 (67%)
CMMLt	3	4.9	4.3	3.5–7.0	3 (100%)

of 18 (72%) patients with MDS in transformation to AML (comprising 15 with RAEBt and 3 with CMMLt) showed telomere reduction and the mean peak TRA was 6.1 kb. Reduction in telomere length occurs, therefore, more frequently and is more marked in MDS in transformation than in RA. It is important to note, however, that many of the patients with RAEBt did not have short telomeres. Similarly, Ohyashiki et al. demonstrated that some, but not all, patients with MDS showed reductions in telomere length following disease progression [26]. Thus, whilst the presence of short telomeres is associated with leukemic transformation of MDS, the correlation is not absolute. It would also be interesting to correlate telomere length with response to therapy. We cannot comment here on this aspect since the great majority of patients were treated with supportive care only.

Approximately 50% of patients with MDS have karyotypic abnormalities present in their bone marrow cells [30,31]. The association between the presence of complex karyotypes in MDS and a poor prognosis has been made by a number of groups [32,35]. Eight patients in the present study had complex karyotypes and we were interested to determine whether the presence of a complex karyotype had any association with telomere length in MDS. Twenty of 36 (55%) patients with either a normal or simple karyotype demonstrated a reduction in telomere length relative to age-matched controls and the mean peak TRA was 7.1 kb. In contrast, 5 of 8 (62%) patients with a complex karyotype showed a reduction in telomere length and the mean peak TRA was 6.1 kb. Patients in both karyotypic groups were represented by all three main FAB sub-types; RA, RAEB, and RAEBt.

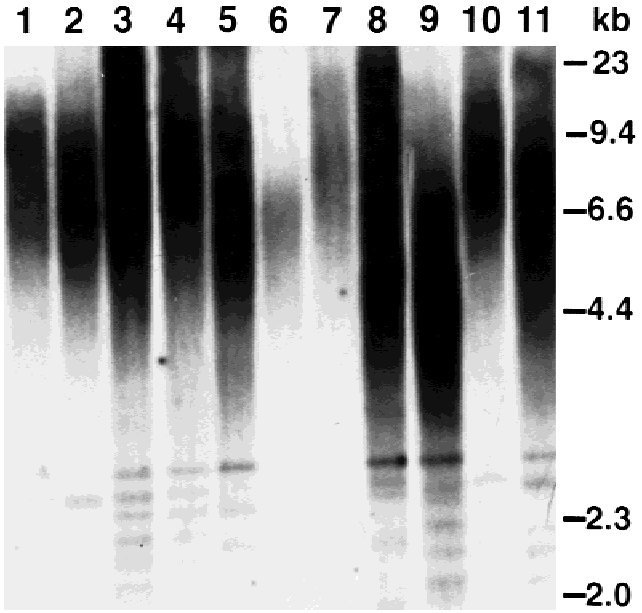

Fig. 1. Southern blot analysis of telomere length using the $(TTAGGG)_4$ probe hybridized against *Hinf*I digested DNA extracted from the bone marrow cells from a group of representative MDS patients. Lanes 1–11 show DNA obtained from the bone marrow of the following patients with MDS (see Table II for case details): 1, case 11 (RA); 2, case 10 (RA); 3, case 9 (RA); 4, case 8 (RA); 5, case 7 (RA); 6, case 6 (RA); 7, case 46 (RAEBt); 8, case 37 (RAEBt); 9, case 54 (CMMLt); 10, case 43 (RAEBt); 11, case 39 (RAEBt). Note heterogeneity of telomere length in MDS samples and marked reduction in telomere length in lane 9, for example. Approximate position of the molecular weight markers in kilobase pairs (kb), is shown on the right-hand side.

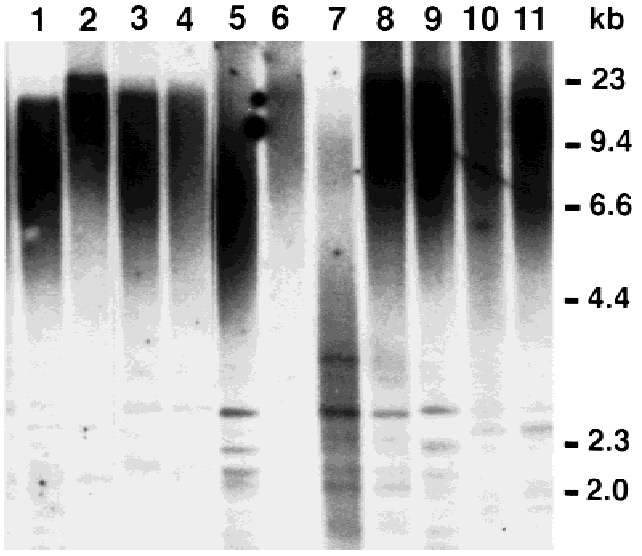

Fig. 2. Southern blot analysis of telomere length using the $(TTAGGG)_4$ probe hybridized against *Hinf*I digested DNA extracted from the granulocyte fraction and lymphocyte fractions of a group of representative MDS patients (lanes 1–8) and the granulocyte fractions of a group of healthy normal individuals (lanes 9–11). The individual lanes show the following: 1 (granulocyte fraction) and 2 (lymphocyte fraction), case 18 (RA); 3 (granulocyte fraction) and 4 (lymphocyte fraction, case 17 (RA); 5 (granulocyte fraction) and 6 (lymphocyte fraction), case 21 (RA); 7 (granulocyte fraction) and 8 (lymphocyte fraction) case 49 (RAEBt); 9–11 (granulocyte fractions) from normal healthy individuals, samples 17, 18, 20, respectively. Note heterogeneity of telomere length in MDS samples and marked reduction in telomere length in the granulocyte fraction (lane 7) of case 49 compared both to the lymphocyte fraction (lane 8) obtained from the same case and the granulocyte fractions (lanes 9–11) of normal healthy individuals, for example. Approximate positions of the molecular weight markers are shown in kilobase pairs (kb) on the right-hand side.

Thus, whilst a reduction in telomere length occurs only slightly more frequently in patients with complex karyotypic abnormalities than in patients with normal or simple karyotypes the observed reduction in peak TRA is more marked in the former and appears to occur irrespective of FAB sub-type. In two studies concerning telomere length in haematological malignancy by Ohyashiki et al. it was reported that the presence of short telomeres often correlated with the presence of a complex karyotype [26,40]. In the study by Ohyashiki et al. regarding telomere length in MDS, 3 of 6 patients with telomere shortening at the time of diagnosis had complex karyotypes whilst 6 of 7 patients with MDS and no significant reduction in telomere length at the time of diagnosis or during disease progression had normal or simple karyotypes [26]. It is possible that this phenomenon is related to genetic instability.

Short telomeres were occasionally observed in patients with normal karyotypes in this present study and conversely patients with complex karyotypes did not always have short telomeres. In the study by Ohyashiki et al. no patients with MDS and normal karyotypes demonstrated telomeric shortening [26]. In the present study, a small number of patients with normal karyotypes did have short telomeres but is most probable that this apparent discrepancy simply reflects the much larger sample number in our study; 22 cases of MDS with a normal karyotype were included in the current investigation as compared to four in that of Ohyashiki et al. [26]. It is important to note that the cases with abnormal karyotypes all had greater than 75% abnormal metaphases on bone marrow analysis. Therefore, from cytogenetic study we have no evidence to support the notion that cases of RA with abnormal karyotypes but with normal telomere length had a ''smaller'' clone than those with a more pronounced shortening of the telomeres.

Thus, in conclusion, there is marked heterogeneity of telomere length in MDS and this is found across the spectrum of FAB sub-types. In contrast to AML, in which consistent shortening of telomeres has been reported by a number of groups, many patients with MDS show telomere lengths identical to age-matched controls.

Furthermore, a marked reduction in telomere length in MDS is often associated with leukemic transformation and with the presence of complex karyotypic abnormalities.

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